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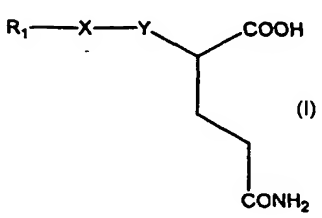
(54) Title: COMPOUNDS AND METHODS FOR INHIBITING AXILLARY MALODOUR



(I)

(57) Abstract: Enzymes mediating in the release of compounds characteristic of human malodour and in particular axillary malodour, and compounds that inhibit said enzymes having general formula (I).

COMPOUNDS AND METHODS FOR INHIBITING AXILLARY MALODOUR

This invention is concerned with methods, compounds and compositions useful for the prevention or suppression of human malodour, in particular human axillary malodour.

It is known that fresh sweat is odourless and that odour is only formed upon contact of sweat with skin bacteria (for example bacteria of the genera of *Staphylococcus* and *Corynebacteria*) and it is believed that odourless molecules present in sweat are degraded by bacteria colonising the axilla. It is generally accepted (Labows *et. al.*, Cosmet. Sci Technol. Ser. (1999), 20:59-82) that highly unpleasant malodour is released from fresh sweat mainly by the *Corynebacteria* genus of bacteria. The principal constituents thought to be responsible for malodour include volatile steroids, volatile sulphur compounds and short-chain, branched fatty acids.

It has been suggested to treat malodour by eradicating the bacteria responsible for causing the odour. Indeed, commercially available cosmetic deodorants often contain antibacterial compounds that generally inhibit the growth of skin microflora. Antibacterial compounds currently used in deodorant products include, for example Triclosan (2,4,4'-trichloro-2'hydroxy-diphenyl-ether). However, a draw-back to the use of antibacterials is the potential for disturbing the equilibrium of the skin's natural microflora.

It has also been suggested to include compounds in a deodorant that would specifically target and suppress the biochemical reactions that transform odourless precursors present in sweat into volatile malodorous steroids or sulphur compounds. Specifically, there have been several publications concerned with the inhibition of enzymes that are thought to be responsible for the release of volatile steroids or volatile sulphur products. In this regard see US patents 5,487,886; 5,213,791 and 5,595,728 which describe amino acid β-lyase inhibitors for use in deodorants. These agents are thought to block the release of sulphur volatiles from cysteine derivatives. US patents 5,676,937 and 5,643,559 describe inhibitors of bacterial exoenzymes, namely sulphatases and glucuronidases. These compounds are supposed to reduce the release of volatile steroids from the corresponding sulphates or glucuronides. Patent application WO 00/01355 describes inhibition of steroid reductases. Finally, in German patent applications DE 19858811A1 and DE 19855956A1 the use of esterase inhibitors as deodorant active ingredients is described.

However, fatty acids, in particular short chain, branched fatty acids are known to play a role in axillary malodour, and are particularly foul smelling. Whereas WO 00/01356 attributes axillary malodour to the catabolism of long-chain fatty acids and teaches the use of certain perfumes to inhibit such catabolism, the art does not reflect an appreciation of the enzymatic process resulting in the release of malodorous fatty acids, in particular short chain, branched fatty acids and therefore does not teach how malodour from these sources may be prevented or suppressed.

The applicant has now discovered the mechanism of the release of fatty acids in sweat and has found an enzyme thought to be responsible for transforming odourless precursor compounds found in sweat, into malodorous fatty acids. The applicant has also found specific inhibitors of the enzyme and screening tools for identifying potential inhibitors, and also methods and compositions for preventing or suppressing malodour. These and other aspects of the present invention will become apparent to those skilled in the art from the following description.

The invention provides in a first aspect an enzyme that mediates in a biochemical process whereby essentially odourless precursor compounds found in sweat are cleaved to release malodorous compounds, particularly malodorous fatty acids, more particularly malodorous short chain, branched fatty acids.

The enzyme of the present invention was isolated from the bacteria of the genus *Corynebacteria* that can be found colonising the axilla, in particular certain *Corynebacteria sp.*, more particularly *Corynebacteria striatum* Ax 20 which has been submitted on the 26, April 2001 to the International Depository Authority DSMZ- Deutsche Sammlung Von Mikrooganismen Und Zellkulturen GmbH, D-38124 Braunschweig. The Accession Number provided by the International Depository Authority is DSM 14267.

The enzyme has not heretofore been available in isolated form. By "isolated" is meant that the enzyme is removed from its original environment, i.e. from the environment in which it is naturally occurring. The present invention therefore provides the enzyme in isolated form, more particularly in isolated, purified form. By "purified form" is meant at least 80%, more particularly greater than 90%, still more particularly 95%, most particularly 99% or greater with respect to other protein and/or nucleic acid contaminants. The enzyme may be characterised by the amino acid sequence set forth in SEQ ID NO: 1. However, also included in the scope of the invention are proteins or polypeptides, e.g. enzymes that comprise amino acid sequences that

are substantially similar to the amino acid sequence as set forth in SEQ ID NO: 1. In its broadest sense, the term "substantially similar" when used in relation to an amino acid sequence, means a sequence corresponding to a reference amino acid sequence, wherein said corresponding sequence encodes for a polypeptide or protein, e.g. an enzyme having substantially the same structure and same function as the enzyme encoded for by the reference amino acid sequence. The percentage identity in sequences may be for example, at least 80%, particularly at least 90% and most particularly at least about 95% of the amino acid residues match over a defined length of the molecule and includes allelic variations. Sequence comparisons may be carried out using a Smith-Waterman sequence alignment algorithm which is known in the art.

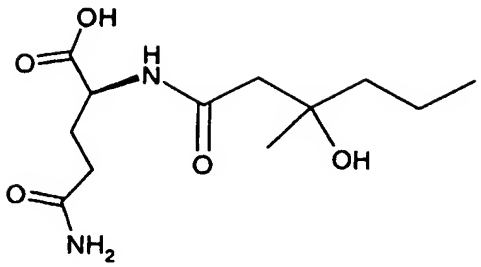
Partial amino acid sequences of the enzyme set forth in SEQ ID NO: 2; NO: 3, and NO: 4 comprise additional aspects of the invention.

The amino acid sequence set forth in SEQ ID NO: 1 may be derived from the open reading frame contained in SEQ ID NO: 5. Accordingly, the invention provides in another of its aspects an isolated nucleic acid, for example set forth in SEQ ID NO: 5, encoding for an enzyme having an amino acid sequence set forth in SEQ ID NO: 1.

All sequence data may be obtained according to techniques commonly known in the art.

An enzyme of the present invention may have a molecular weight of 43 to 48 kDa. In particular, it may have an apparent molecular mass on SDS-PAGE of 48 kDa and an effective molecular mass of 43365 Da as determined by nano-ESI MS (electron spray ionisation mass spectrometry) analysis and also derived from the amino acid sequence.

An enzyme of the present invention mediates in a biochemical process whereby essentially odourless precursor compounds are cleaved to release malodorous compounds characteristically found in sweat. The precursor compounds are substrates that may generally be described as derivatives of L-glutamine, in particular L-glutamine derivatives wherein the N_α atom of the L-glutamine residue is acylated with a residue of a malodorous compound, in particular a fatty acid residue, more particularly a short chain, branched fatty acid residue. One example of such a precursor compound that was isolated from human sweat has the structure:



Cleavage of this substrate at the N_α position releases the 3-hydroxy-3-methyl-hexanoic acid, itself having a pungent odour, which may dehydrate to give 3-methyl-3-hexenoic acid which is another key malodour volatile in human sweat. Proteins or polypeptides, e.g. enzymes that act to cleave substrates of the type referred to hereinabove to release malodorous acids are within the ambit of the present invention.

An enzyme according to the present invention may be particularly active in relation to certain substrates. For example, it can recognise N_α-acylated-L-glutamine substrates. However, it is not able to cleave similar acylated derivatives of related amino acids such as L-glutamate, L-aspartate or L-asparagine; nor does it recognise substrates wherein the N_δ or the COOH group of the L-glutamine moiety is substituted. Furthermore, it is stereospecific, for example it recognises derivatives of L-glutamine and not the analogues derived from D-glutamine. Having regard to the substrate specificity, an enzyme of the present invention may be described as an aminoacylase, more particularly, an N_α-acyl-glutamine-aminoacylase. The acyl group at the N_α atom can vary widely, and the enzyme may cleave substrates for a wide variety of different smelling and non-smelling acids and other compounds. It may also, in addition to amide bonds, cleave carbamate bonds at the N_α position thereby mediating in the release of an alcohol, CO_2 and L-glutamine. It may also cleave acylated derivatives of L-glutamine where the N_α atom has been replaced by an oxygen atom, i.e. oxo-glutamine-derivatives.

Further, the enzyme requires as a cofactor a zinc ion. In this respect and in its ability to cleave amide-bonds, it may be considered to be related to the group of enzymes known as zinc-metallopeptidases. More specifically, since it may cleave an amide-bond situated next to a terminal carboxyl group, it may also be considered to be related to the group of enzymes known as the zinc-carboxypeptidases.

Whereas an enzyme of the present invention is highly selective for the glutamine residue of a substrate, as mentioned above, applicant has surprisingly found that a wide variety of glutamine derivatives are able to fit into the enzyme. For example applicant found that disparate substrates such as Nα-(3-hydroxy-3-methyl-hexanoyl)-L-glutamine, Nα-(3-methyl-2-hexenoyl)-L-glutamine, Nα-lauroyl-L-glutamine, Nα-(11-undecenoyl)-L-glutamine, Nα-tetradecanoyl-L-glutamine, Nα-decanoyl-L-glutamine, Nα-phenylacetyl-L-glutamine, Nα-Carbobenzyloxy-L-glutamine (=Z-glutamine), Nα-3,7-Dimethyl-6-octenyloxycarbonyl-L-glutamine, Nα-(3-hexenyl)oxycarbonyl-L-glutamine, Nα-Butyloxycarbonyl-L-glutamine, Nα-(4-tert-Butylcyclohexyloxycarbonyl)-L-glutamine, Nα-2-Phenylethyloxycarbonyl-L-glutamine, Nα-(3-Methyl-5-phenylpentanoxycarbonyl)-L-glutamine, Nα- (2-Adamantan-1-yl-ethoxycarbonyl)-L-glutamine, Nα-(2-Adamantan-1-yl-methoxycarbonyl)-L-glutamine, Nα-[2-(2,2,3-trimethyl-cyclopent-3-enyl)-ethoxycarbonyl)-L-glutamine, and Nα-(4-methoxy-phenylsulfanylcarbonyl)-L-glutamine are all able to be cleaved by enzyme. These findings, together with knowledge as to the nature of metallopeptidases, suggest that an enzyme of the present invention has a high specificity for glutamine at its so-called "S_1'-site", but will accept a wide variety of substituents at the Nα-atom of the substrate provided those substituents are sufficiently bulky and hydrophobic to be received into the so-called "S_1 site" of the enzyme. The terms "S_1 site" and "S_1' site" as used herein relate to the sites on metallopeptidase enzymes as will be apparent to a person skilled in the art.

An enzyme described hereinabove represents a particularly preferred embodiment of the present invention. However, other bacterial strains, for example other strains of *Corynebacteria,* or bacteria of the genus *Staphylococci* found in the microflora of the axilla also produce related enzymes that themselves mediate in biochemical reactions wherein L-glutamine derivatives are cleaved at N_α. However, these related enzymes specifically cleave precursor compounds to release straight chain fatty acids, which acids play only a minor role in typical axilla malodour. These related enzymes, and inhibitors thereof, also form embodiments of the present invention.

A further aspect of the invention comprises a method of isolating an enzyme described above. Enzyme of the present invention occurs intracellularly and can be released from the cells by mechanical disruption of the cell envelope. Thus, an enzyme may be isolated from cellular extracts obtained from wild-type bacterial strains, especially from strains of Corynebacteria isolated from the human axilla, in particular Corynebacterium striatum Ax 20.

Alternatively, an enzyme may be manufactured by recombinant means and the invention provides in another of its aspects such methods, recombinant vectors and their use as reagents in said manufacture, and procaryotic or eurocaryotic host cells transformed with said vectors.

Thus, an enzyme may be produced by growing host cells transformed by an expression vector comprising foreign nucleic acid that encodes for the enzyme under conditions such that it is expressed, and thereafter recovering it according to known techniques. In a particular embodiment of the invention a nucleic acid fragment that encodes for the SEQ ID NO: 5 or a substantially similar nucleic acid sequence coding for an enzyme with an amino acid sequence which is substantially the same as sequence SEQ ID NO 1, is introduced into an expression vector by operatively linking the nucleic acid to the necessary expression control regions required for gene expression. The vector is then introduced into an appropriate host cell, e.g. a bacterial host cell, more particularly E.Coli. Numerous expression vectors are known and commercially available, and the selection of an appropriate expression vector and suitable host cells which they can transform is a matter of choice for the skilled person. Examples of expression vectors and host strains are described in T. Maniatis et al. (Molecular Cloning, cold spring Harbor Laboratory, 1982), other examples of vector-host strain combinations are the vector pPROTet.E133 in strain DH5$_\alpha$PRO which may be obtained from Clontech (Palo Alto, California, USA) or the vector pBADgIIIA in strain TOP 10, which may be obtained from Invitrogen (Groningen, The Netherlands).

Recombinant production of an enzyme according to the invention is not limited to the production in bacterial hosts. Any other means known to those skilled in the art of producing an enzyme based on a defined genetic sequence may be used. Such methods include, for example the expression in genetically modified yeasts, in insect cells transformed with a modified baculovirus and in eucaryotic cell lines or the *in vitro* transcription and translation.

The enzyme produced according to methods described above may be purified according to known techniques. Thus, host-cells containing the enzyme may be extracted to release the enzyme, e.g. by mechanical disruption of the cells or by osmotic shock. Thereafter, crude enzyme may be separated from host cell debris and host cell protein and nucleic acid contaminants using well known techniques such as precipitation and chromatography. Any of the chromatography techniques known in the art for purifying proteins may be employed. For

example, ion-exchange, hydrophobic interaction, reverse phase, and size exclusion chromatography steps may be employed in any suitable sequence. Optionally, after each chromatography step the eluted enzyme may be further purified by filtration and concentrated using, e.g. ultrafiltration techniques.

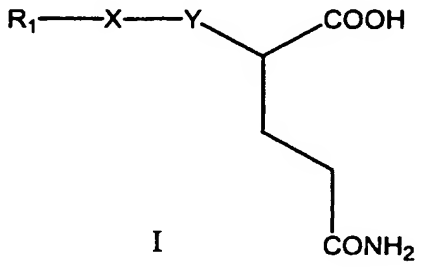
In another aspect of the invention there is provided a method of screening compounds as inhibitors of an enzyme as hereinabove described. In particular, in order to identify inhibitory compounds, the enzyme or cells or cell extracts containing the enzyme, obtained from any of the above described sources, may be incubated along with an appropriate substrate that is cleavable by the enzyme, and with potential inhibitory compounds. An appropriate substrate may be selected from any of the class of precursor compounds referred to hereinabove, in particular an N_α-acylated L-glutamine or carbamate of glutamine. Particular useful substrates are N_α-3-methyl-3-hydroxy-hexanoyl-glutamine, N_α-lauroyl-L-glutamine (commercially available from Fluka, Buchs, Switzerland) and N_α-carbobenzyloxy-L-glutamine (Z-glutamine; commercially available from Aldrich, Buchs, Switzerland). After a certain time of incubation, which may be determined according to routine experimentation, analysis may be performed by measuring the released acid or alcohol, or by measuring the amount of free L-glutamine. A particularly useful approach for high-throughput screening of potential inhibitors may be to measure the release of free L-glutamine by derivatising the free N_α group with an amine-group derivatising agent, which upon reaction with the amine group forms a chromophore or a fluorescent molecule. Particularly useful in this regard may be the use of fluorescamine (commercially available from Fluka, Buchs, Switzerland) to form a fluorescent molecule upon reaction with L-glutamine. Finally, the cleavage of the L-glutamine-substrate may be compared to control reactions and the potential of the test compounds to inhibit the reaction may thereby be quantified.

Having regard to the nature of the enzyme and the screening method set forth above the skilled person will be able to derive compounds that are inhibitors of the enzyme in its mediation in the biochemical reaction resulting in the release of malodorous compounds, and these inhibitors form yet another aspect of the invention.

Potential inhibitors may be selected, by way of non-limiting example, from dithiols, which molecules are capable of strongly co-ordinating to an active-site zinc atom located on the enzyme. One example of such a compound is dithiothreitol (2,3-dihydroxy-butane-1, 4-dithiol). Other zinc chelators may be useful inhibitors; such chelating agents may include o-

phenanthroline, EDTA, Na–pyrithione, amino-tri(methylene-phosphonic acid), ethylene-diimino-dibutyric acid (EDBA), Ethylenediamine-2-2'-diacetic acid, pyridine-2,6 dicarboxylic acid, Diethylenetriamine pentaacetate, Ethylenediamine disuccinic acid, and N,N,N',N'-tetrakis-(2-pyridylmethyl)-ethylenediamine. A further group of inhibitors may be selected from Nα-acyl-L-glutamines or carbamates of L-glutamine which introduce some steric hindrance into the moiety substituted at the N_α atom. These inhibitors may compete with the natural precursor compounds found in sweat for the active zinc site on the enzyme, but display a reduced tendency, or no tendency, relative to the natural precursor compound, to cleave at the N_α atom.

Compounds of formula (I)

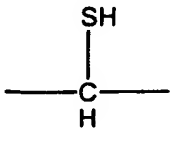


I

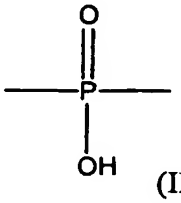
have been found to be particularly interesting inhibitors of the enzyme and these compounds form a preferred embodiment of the present invention.

In formula (I), Y represents a direct bond to X, or a divalent chain that may contain carbon, oxygen or nitrogen atoms, and may comprise functionality such as amide functionality –CONH- provided that the chain is not cleavable by the enzyme under condition of use. Preferably this divalent chain contains no more than 3, and preferably no more than 2 atoms in the chain.

X represents a zinc-chelating group, e.g. a group bearing carboxylic acid functionality, or more particularly a methylene thiol group (II), or a phosphinyl group (III)



(II)



(III)

As regards the group R_1, given the broad range of substituents that can fit into the S_1 site of the enzyme, the nature of this group may vary widely provided it is sufficiently hydrophobic and/or bulky to fit into this site. Preferably, it represents a linear, branched or cyclic carbon chain having about 1 to 14 carbon atoms, more particularly about 4 to 14 carbon atoms. The afore-mentioned chain may contain one or more heteroatoms such as O, N or S, and it may also contain unsaturation. The chain may support one or more substituents, for example amide, ester, keto, ether, amine or hydroxyl halogen, or aryl or heteroaryl substituents which aryl or heteroaryl groups may support substituents selected from amide, ester, keto, ether, amine, halogen, alkyl or hydroxyl. The term "aryl" or "heteroaryl" as used herein is preferably a mono-cyclic or polycyclic group containing from 6 to 14 carbon atoms, and as appropriate one or more heteroatoms such as O, N or S. By way of example, any of the substituents attached to the acyl carbonyl group of the substrates mentioned above would be suitable as a group R_1.

More preferred groups R_1 may be selected from a C_{1-14} alkyl or C_{2-14} alkenyl, more preferably a C_{4-14}alkyl or alkenyl, e.g. n-butyl or sec-butyl, or an alkyl or alkenyl group here-mentioned substituted with a phenyl group, or a phenyl group substituted with any of the substituents referred to above, e.g. a benzylic group or a phenylethyl group.

More preferred compounds of formula (I) are those compounds wherein

Y is selected from a direct bond to X, C_{1-3} alkylene, e.g. methylene, –CONH-, or –NH-, and

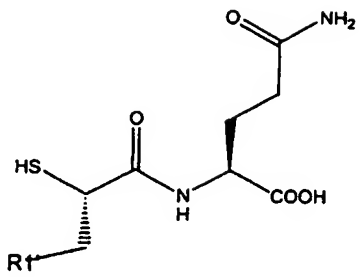
X is selected from methylene thiol (II) or phosphinyl (III).

Most preferred compounds of the present invention are those compounds of formula (I) wherein

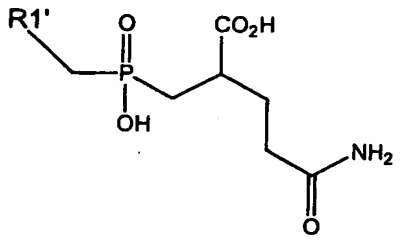
Y represents an amide group –CONH- when X is methylene thiol (II) or Y represents a methylene group when X is phosphinyl (III).

Compounds of formula (I) contain chiral atoms and as such they can exist as diastereomeric mixtures or they may exist as pure stereo-isomers. Most preferred compounds have an S – configuration on the Glutamine moiety, and in the case of the methylene-thiol containing compounds also an S-configuration at the chiral centre in this group is preferred.

Examples of most preferred compounds are



wherein R1' is phenyl (5a); iso-C_3H_7 (5b); or n-C_3H_7 (5c); or,



wherein R1' is phenyl (8a); iso-C_3H_7 (8b); or n-C_3H_7 (8c).

The N-(sulfamylacyl)amino acids exemplified as (5a-c) above may be prepared in a manner set forth in Scheme I of Figure 1. The t-butyl ester of glutamine may be coupled in a classical procedure of liquid-phase peptide synthesis using EDCI + HOBT with various acetylsulfamyl alkanoic acids (2) leading to (3). After deprotection of the t-butyl ester group and hydrolysis of the acetylsulfamyl group, one may obtain the compounds (5). The various acetylsulfamyl alkanoic acids (2) may be obtained form the corresponding alpha amino acids. Brominative deamination leads to the removal of the alpha-amino functionality replacing it with a bromine atom with retention of configuration. Subsequent removal of the bromine atom with the potassium salt of thioacetic acid will provide a compound (2) with inversion of configuration.

The skilled person will appreciate that other N-acylated glutamine compounds of the present invention may be synthesised in an analogous manner using appropriate reagents to provide the desired R_1-X-Y-residue.

The phosphinic-type compounds may be prepared by condensation of a desired alkyl halide and phosphinic acid ammonium salt to give a compound (6), followed by a 1,4 addition of (6) to the ethyl-2-(N-trityl)carboxamidoethyl acrylate as set forth in Scheme 2 of Figure 2. Deprotection of the ester group and the amide can be achieved in manner known per se to form the desired compound. The acrylate compound may be formed according to a method set forth in Scheme 3 of Figure 3. The skilled person will appreciate that other compounds of the present invention can be produce by 1,4 addition of a residue bearing R_1-X-Y- with the aforementioned acrylate.

Further details regarding the synthesis of compounds of the present invention are disclosed in the Examples hereinbelow.

Applicant has disclosed a wide range of compounds with inhibitory properties. However, having regard to the wide substrate specificity of the enzyme of the present invention responsible for the release of the malodorous compounds found in sweat, and the reliable methodology for identifying inhibitors of the enzyme, the applicant is able to provide a novel method in the suppression of body odours which methods form an additional aspect of the invention.

Accordingly, the invention provides in another of its aspects, a method of suppressing axillary malodour comprising the step of providing a composition for application to a person in need of treatment, said composition containing an inhibitor compound and dermatologically acceptable vehicle therefor, said compound being selected from a screening of compounds for activity in the inhibition of the enzyme.

Compounds, which inhibit an enzyme of the present invention reduce the activity of the enzyme and may lead to a significant reduction of the release of malodour acids from odourless fresh sweat. Compounds of the present invention display inhibition of the enzyme at concentrations ranging from 10^{-3} to 10^{-8} Molar. The activity of the compounds as inhibitors may be measured in terms of either their IC_{50} values or their Ki values, both of which measures are well known to the person skilled in the art. As is well known, the IC_{50} value provides the concentration of an inhibitor needed to reduce enzyme velocity by half at a given substrate concentration. This value is dependent on the affinity of the substrate for the enzyme which is reflected in the value K_m of the substrate. In this way, the Ki value may be determined for a given substrate and a given

substrate concentration by measuring IC_{50} and then calculating according to the following formula

$$K_i = \frac{IC_{50}}{1 + \frac{[Substrate]}{K_m}}$$

Ki values for certain preferred inhibitors are set forth in Example 8 below.

Compounds of the present invention may be added to any cosmetic and personal care products such as sticks, roll-ons, pump-sprays, aerosols, deodorant soaps, powders, solutions, gels, creams, sticks, balms and lotions to enhance the deodorising effect of these products. Preferably, a compound of the present invention may be employed in said products in amounts of about 0.01 to 0.5% by weight.

The above-mentioned products, in addition to the inhibitors, may comprise anti-bacterial agents known in the art, e.g. Triclosan. The products may also comprise dermatologically acceptable ingredients such as are commonly used in these types of product. Examples of such additional ingredients include fragrances, colorants, opacifiers, buffers, antioxidants, vitamins, emulsifiers, UV absorbers, silicones and the like. As is also well known, all products can be buffered to the desired pH.

In addition to the inhibitor, a deodorant cologne may comprise ethanol and fragrance. Fragrance may be present from 1 to 10% and the ethanol can be present to make up the mass to 100%.

Additional ingredients in a typical ethanol-free deodorant stick may include polyols, such as propylene glycol; derivatives thereof, such as propylene-glycol-3-myristyl ether (Witconol APM); water; a surfactant such as sodium stearate; and a fragrance. The polyol may be present in an amount of 30 to 40%; the derivatives of the polyol likewise may be present at about 30 to 40%; water may be present to about 10 to 20%; the surfactant may be present to 5 to 10%; and the fragrance may be present in an amount mentioned above.

A typical antiperspirant stick might contain as additional ingredients such as Ethylene Glycol Monostearate (e.g. from 5 to 10%); Shea butter (e.g. from 3 to 5%); Neobee 1053 (PVO International) (e.g. fromabout 12 to 15%); Generol 122 (Henkel) (e.g. from about 3 to 7%);

Dimethicone (DC 345)(e.g. from 30 to 40%); aluminium sesquichlorohydrate (e.g. from about 15 to 20%); and a fragrance, e.g. from 1 to 10%.

An antiperspirant aerosol may contain ethanol, e.g. from about 10 to 15%; zirconium aluminium tetrachlorohydrate, e.g. from about 3 to 5%; Bentone 38, e.g. from about 1 to 2%; fragrance in an amount aforementioned; and a hydrocarbon propellant, e.g. S-31 up to 100%.

An antiperspirant pump composition may contain aluminium sesquichlorohydrate, e.g. from 15 to 25%; water, e.g. from 50 to 60%; Triton X-102 (Union carbide), e.g. from 1 to 3%; dimethyl Isosorbide (ICI), e.g. from 15 to 25 %; and a fragrance in an amount as aforementioned.
All percentages mentioned above are in wt %.

Accordingly, the present invention relates to the use of inhibitor compounds in compositions for the elimination or suppression of malodour. The invention also relates to compositions comprising an odour suppressing quantity of an inhibitor of the enzyme and dermatologically acceptable vehicles which are generally well known in the art of cosmetic and personal care products.
In an alternative method of malodour prevention or suppression, instead of, or in addition to, employing inhibitors that act to prevent or suppress the activity of the enzyme, one may employ agents that reduce the expression of the enzyme in bacteria containing a gene coding for said enzyme. Such agents may be screened either using wild-type strains or genetically engineered strains of the bacteria expressing the enzyme. If wild-type strains are used, the level of enzyme expression may be directly measured under various environmental conditions and upon addition of potential inhibitory compounds. Alternatively, genetically engineered Corynebacteria that are transformed with a vector containing a reporter gene may be used. These vectors may contain the reporter gene under the control of the regulatory sequences for the enzyme expression, which regulatory sequence is contained in the SEQ ID NO: 6 and which forms another aspect of the invention. For this purpose, the regulatory sequence, or any part thereof, may be cloned upstream of the reporter gene into a broad host-range vector able to transform Corynebacteria. The reporter gene may thereby be put under the regulatory control of the genetic sequence which controls the expression of the enzyme. The vector obtained in this way may then transformed into a strain of Corynebacterium. Particularly useful vectors for this purpose are described by M.P. Schmitt (Infection and immunity, 1997, 65(11): 4634-4641) and by N. Bardonnet and C. Blanco (FEMS Microbiol Lett., 1991, 68(1):97-102). Particularly useful

marker genes are lacZ (coding for β-galacturonidase, gfp (coding for the green fluorescent protein), luxABCD (coding for bacterial luciferase) and gusA (coding for glucuronidase). The genetically engineered strain may then be grown in the presence of a compound to be tested and the expression of the marker gene may be measured by conventional methods. Compounds that lead to a reduction in expression (i.e. reduce the level of mRNA) may reduce malodour formation by reducing the level of enzyme in the axilla.

There now follows a series of examples that serve to illustrate the invention.

Example 1

Isolation of new malodour acid and precursors thereof from human sweat

Fresh axilla secretions were sampled from human panellists by washing the axilla with 10% ethanol. The samples were extracted with MTBE to remove interfering lipids. The hydrophilic phases obtained from the washings from several individuals were then pooled. This material was practically odourless, but upon hydrolysis of sub-samples with 1 M NaOH, it produced typical axilla malodour. To identify the malodour volatiles, hydrolysed sub-samples were extracted and concentrated by solid phase extraction and then analysed by GC-sniff. Peaks that were rated as having a strong odour and closely related to axilla malodour were analysed by GC-MS. The samples contained one particular peak of an acid very typical of axilla malodour. Based on the MS data the most probable structure of this peak was 3-hydroxy-3-methyl-hexanoic acid. This assumption was verified by synthesising this latter compound and comparing its spectra and retention times to the GC-MS data of the major malodour peak in the GC-sniff analysis. This new malodour compound is structurally related to the known sweat malodour acid 3-methyl-2-hexenoic acid, and it is transformed into this latter compound by dehydration upon prolonged incubation.

To identify the precursor for this acid, the pooled non-hydrolysed sample was separated on a Superdex gel filtration column (Pharmacia, Uppsala, Sweden) using NH_4CO_3/NaCl as the elution buffer. Individual fractions of this separation step were tested for the content of a malodour precursor by hydrolysis with 1 M NaOH. One fraction developed strong malodour upon hydrolysis and this malodour could be attributed to the release of 3-hydroxy-3-methyl-hexanoic acid by GC-MS analysis. This fraction was subjected to LC-MS analysis. It contained one major mass peak of 274 Da and an additional peak at 256 Da. The mass spectrum of the

former peak suggested a compound where the 3-hydroxy-3-methyl-hexanoic acid is linked to one molecule of L-glutamine (i.e. N_α-3-hydroxy-3-methyl-hexanoyl-L-glutamine), while the second peak could, based on its mass, correspond to the dehydrated analogue N_α-3-methyl-2-hexenoyl-L-glutamine. N_α-3-hydroxy-3-methyl-hexanoyl-L-glutamine was then synthesised and its MS spectrum and retention time in the LC-MS-analysis compared to and found identical with the compound isolated from natural sweat.

Example 2

Isolation of axilla bacteria having the ability to cleave the malodour precursor compound

The axillary flora of 8 panellists was isolated with the detergent-scrub method: A 6 cm^2 area of the axilla was scrubbed with a phosphate buffer at pH 7 containing 1% Tween 80. The samples were spread-plated on tryptic soy agar amended with 5g/L of Tween 80 and 1 g/L of lecithin. Single isolates obtained after 48 h incubation were subcultured and characterised. A total of 24 individual strains were identified based on colony and cell morphology, gram-reaction, lipophilic growth, lipase reaction and API identification kits (bioMerieux, France; coryneforms with the API coryne kit and cocci with the ID Staph 32 kit). The strains were grown overnight in a liquid medium (Mueller-Hinton amended with 0.01% Tween 80), harvested by centrifugation and resuspended to a final OD_{600} of 1 in a semi-synthetic medium (Per litre: 3 g KH_2PO_4, 1.9 g K_2HPO_4, 0.2 g yeast extract, 0.2 g $MgSO_4$, 1.4 g NaCl, 1 g NH_4Cl, 10 mg $MnCl_2$, 1 mg Fe_3Cl_2, 1 mg $CaCl_2$). Aliquots of this stationary culture were then amended with a final concentration of 500ppm of N_α-3-hydroxy-3-methyl-hexanoyl-L-glutamine (5% stock solution dissolved in methanol). After 24 h incubation (with shaking at 300rpm; 36°C) the samples were extracted and the amount of released 3-hydroxy-3-methyl-hexanoic acid was determined by capillary GC. Table 1 gives the results for a subset of the strains tested. From these results it appears that among the *Corynebacteria* isolated from the axilla some, but not all, are able to release 3-hydroxy-3-methyl-hexanoic acid form the synthetic precursor. The *Corynebacteria* which are able to conduct this biochemical reaction may be found in the group of the lipophilic and in the group of the non-lipophilic *Corynebacteria*. Therefore, a specific enzyme only present in some bacterial strains seems to be responsible for this cleavage. Since it releases axilla malodour the putative enzyme was named AMRE, which stands for 'axillary malodour releasing enzyme'. Apparently the tested *Staphylococci* are not able to catalyse this reaction, which is in agreement with the observation, that only subjects with an axilla flora dominated by *Corynebacteria* produce the most typical axilla malodour (Labows *et. al.*, Cosmet. Sci Technol. Ser. 1999,

20:59-82). However, when Nα-lauroyl-L-glutamine was used as substrate in the same experiment, it was found that also other *Corynebacteria* and some *Staphylococci* can release lauric acid from this substrate. It therefore appears, that most axilla bacteria have a related enzyme, but that many can only release straight fatty acids which make a minor contribution to typical axilla malodour.

Table 1. Cleavage of the natural malodour precursor by axilla bacteria.

Isolate	Species assignment	Lipophilic (*)	3-hydroxy-3-methyl-hexanoic acid released (ppm)
Ax1	*Staphylococcus capitis*	-	0
Ax6	*Staphylococcus epidermidis*	-	0
Ax9	*Micrococcus luteus*	-	0
Ax3	*Corynebacterium bovis*	+	0
Ax7	*Corynebacterium group G*	+	0
Ax15	*Corynebacterium jeikeium*	+	37.4
Ax19	*Corynebacterium jeikeium*	+	105.1
Ax20	*Corynebacterium striatum*	-	262.7

(*) Corynebacteria isolated from the human axilla may be separated into two classes based on their requirement for a source of fatty acids in the growth medium.

Example 3

Purification and analysis of the enzyme from strains that cleave malodour precursor compounds

Corynebacterium striatum Ax20 was selected to isolate and purify the enzyme responsible for the cleavage of the precursor Nα-3-hydroxy-3-methyl-hexanoyl-L-glutamine. The strain was grown during 48 h in Mueller-Hinton broth amended with 0.01% Tween 80. A total volume of 2 L of culture was harvested by centrifugation. The pellet was washed in Buffer A (50mM NaCl; 50 mM NaH_2PO_4/K_2HPO_4 buffer at pH 7) and this buffer was used throughout the whole purification procedure. The cells were disrupted mechanically by vortexing them with glass beads (425-600 µm, Sigma, St-Louis, USA) during 30 min at maximal speed. The crude cell lysate was then fractionated by precipitation with an increasing concentration of $(NH_4)_2SO_4$. The precipitate obtained between 50% and 80% saturation of $(NH_4)_2SO_4$ contained the active enzyme. This enriched sample was dissolved in Buffer A and then sequentially passed over four chromatography columns: DEAE Sepharose CL-6B anion exchange resin (Pharmacia, Uppsala, Sweden; elution with a linear gradient from 0 to 800 mM KCl); Phenyl-Sepharose hydrophobic

interaction resin (Pharmacia; elution with a linear gradient from 1000 mM to 0 mM of $(NH_4)_2SO_4$; Mono Q strong anion exchange column on the FPLC system (Pharmacia; elution with a gradient from 0 to 800 mM KCl) and finally Mono P weak anion exchange column on the FPLC (elution with a gradient from 0- 800mM KCl in a 50 mM Bis-Tris buffer instead of Buffer A). After each column separation the active fractions (determined by fluorescent activity assay with Nα-lauroyl-L-glutamine as substrate, see example 8) were pooled and then desalted and concentrated by ultrafiltration (Amicon membrane YM10, Millipore, Bedford, US). The resulting active fractions after the last column separation contained one major protein band with an apparent molecular weight of about 48kDa as determined by SDS-PAGE. Its effective molecular mass was determined by nano-ESI MS analysis and found to be 43365±5 Da. This enzyme retained all its activity if incubated with PMSF (Phenylmethylsulfonylfluoride, Roche Biochemicals, Mannheim, Germany) and Pefabloc SC (4-(2-aminoethyl)-benzenesulfonylfluoride, Roche Biochemicals), which are typical inhibitors for serin- and cystein proteases. On the other hand it was completely inhibited by 1 mM of EDTA and o-phenantrolin. This inhibition could be reversed by the addition of 1mM $ZnCl_2$. This indicates that the enzyme belongs to the class of zinc-dependent metallo-peptidases, requiring a Zn atom as cofactor. Finally, the enzyme was subjected to LC-ESI-MS/MS analysis after tryptic digestion and to analysis of its N-terminal amino acid sequence. This led to identification of its N-terminal amino acid sequence (SEQ ID NO: 2) and to the sequence of two internal peptides (SEQ ID NO: 3; SEQ ID NO: 4).

Example 4

Substrate specificity of the enzyme

To understand in detail the structural requirements of substrates, the enzyme extracted from *Corynebacterium striatum* Ax20 was incubated with a wide variety of said compounds related to the originally isolated N_α-3-methyl-3-hydroxy-hexanoyl-L-glutamine present in sweat. Each compound was used at a concentration of 500ppm in Buffer A, and analysis of released acid or alcohol was done by capillary GC after 24 h of incubation. First, different modifications at the N-terminus were tested. It was found, that the enzyme can cleave such simple substrates as N_α-lauroyl-L-glutamine and N_α-carbobenzyloxy-L-glutamine (=Z-glutamine). From the latter it releases benzyl-alcohol. Other N-lauroyl-amino acids and Z-amino acids (all obtained from Fluka and Aldrich, Buchs, Switzerland) were thus tested, but it was found that among the 20 amino acids occurring in proteins, the enzyme only cleaves L-

glutamine derivatives, and, to much lesser extent, L-alanine derivatives. The results of some of the substrates tested are summarised in Table 2. Furthermore the enzyme can cleave other carbamates of L-glutamine, also derivatives where the alcohol is a fragrance alcohol (for example citronellol, see Table 2 compound 5), and it can therefore be used to release pleasant smelling molecules from precursors. Indeed, it has broad specificity for substituents at N_α as reflected in compounds 1-5 (below) and as discussed above. Finally, it is stereospecific and cannot cleave derivatives of D-glutamine (Table 2, compound 19), it requires a free COOH group of the L-glutamine and does not cleave derivatives in which this group is linked to methanol or glycin (Table 2, compounds 20 and 21). It also cannot cleave a derivative in which the N_δ of glutamine is further derivatised (Table 2, compound 22).

Table 2. Substrate specificity of the enzyme

	Substrate	Cleavage by enzyme[1]
1	Nα-(3-hydroxy-3-methyl-hexanoyl)-L-glutamine	++
2	Nα-lauroyl-L-glutamine	+++
3	Nα-decanoyl-L-glutamine	+++
4	Carbobenzyloxy-L-glutamine	++
5	N_α-3,7-Dimethyl-6-octenyloxycarbonyl-L-glutamine	+++
6	N-Lauroyl-L-aspartate	-
7	Nα-Lauroyl-L-lysine	-
8	Nα-Lauroyl-L-arginine	-
9	N-lauroyl-L-alanine	+
10	Carbobenzyloxy-L-alanine	+
11	Carbobenzyloxy-L-glutamate	-
12	Carbobenzyloxy-L-asparagine	-
13	Carbobenzyloxy-L-aspartate	-
14	Carbobenzyloxy-L-serine	-
15	Carbobenzyloxy-L-tyrosine	-
16	Carbobenzyloxy-L-glycine	-
17	Carbobenzyloxy-L-histidine	-
18	Carbobenzyloxy-L-leucine	-
19	Carbobenzyloxy-D-glutamine	-
20	Carbobenzyloxy-L-glutamine-O-Me	-

21	Carbobenzyloxy-L-glutamine- Gly-OH	-
22	N_δ-benzyl-N_α-carbobenzyloxy-L-glutamine	-

[1] - indicates no cleavage, + indicates cleavage < 10%, ++ cleavage 10 –50% and +++ cleavage over 50%.

Example 5

Isolation of the gene coding for the enzyme

Based on the partial amino acid sequence analysis (see example 3), degenerated primers were designed and used to amplify a 350 bp and a 650 bp fragment of the corresponding gene between the N-terminus (SEQ ID NO 2) and the two internal peptide sequences (SEQ ID NO 3 and 4). Chromosomal DNA of Ax 20 served as template. The primer with the sequence SEQ ID NO 7 successfully annealed at the sequence coding for the N-terminus and the primers with the sequence SEQ ID NO 8 and SEQ ID NO 9 annealed within the sequences coding for the internal peptides. Standard PCR conditions were used, and the annealing temperatures were optimised on a gradient cycler (T-Gradient, Biometra, Göttingen, Germany). The amplified DNA was cloned into the vector pGEM-T Easy (Promega, Madison, USA) and the nucleotide sequence determined on the ABI-Prism model 310 (PE Biosystems, Rotkreuz, Switzerland) using standard methods. Based on the obtained sequence, specific nested oligonucleotides were designed to clone the upstream (SEQ ID NO 10 and 11) and downstream region (SEQ ID NO 12 and SEQ ID NO 13). Chromosomal DNA of Ax 20 was digested with *Sma*I and *Pvu*II and ligated to the GenomeWalker Adaptor (Clontech Laboratories, Palo Alto, USA). The upstream and downstream regions were then amplified as described in the instructions to the Universal GenomeWalker™ kit (Clontech Laboratories, Palo Alto, USA), cloned into the vector pGEM T-easy and the nucleotide sequence determined. With the two enzyme digests two upstream (450 bp and 1200 bp) and two downstream fragments (1200 bp and 3000 bp) were obtained. The full coding sequence of the enzyme (SEQ ID NO 5) as well as upstream (SEQ ID NO 6) and downstream regions were contained in the cloned region. The deduced amino acid sequence of the open reading frame corresponding to the enzyme (SEQ ID NO 1) was compared to public protein sequence databases (Swissprot and GeneBank, bacterial sequences) and it was found to align very well to known aminoacylases, some carboxypeptidases and various putative peptidases identified in genome sequencing projects. A number of these enzymes are

summarised into the peptidase family m40, also known as the ama/hipo/hyuc family of hydrolases.

Example 6

Heterologous expression of the gene coding for the enzyme

The full-length sequence of the open reading frame coding for the enzyme was amplified with PCR from chromosomal DNA of Ax20 using specific primers (SEQ ID NO 14 and SEQ ID NO 15). The amplified DNA fragment was then digested with the restriction enzymes NcoI and Hind III It was then ligated into the vector pBADIIIA (Invitrogen, Groningen, The Netherlands) pre-digested with the same enzymes. The resulting plasmid pBADgIIIAMRE was transformed into the host strain *E. coli* TOP10 (Invitrogen). This strain was grown in LB broth until it reached an optical density of about 0.5 at 600nm. The culture was induced with arabinose (0.2% final concentration) incubated for 4 h, harvested by centrifugation and disrupted by ultrasonication. Enzyme assays with Na-lauryl-Glutamine as substrate were performed in Buffer A with an incubation time of 1h and a substrate concentration of 500ppm. Table 3 gives the activity of extracts obtained from wild-type cells and from extracts of the induced and non-induced modified strains expressing the enzyme.

Table 3. Heterologous expression of the enzyme in *E. coli*

	release of lauric acid from Lauryl-Glutamine, 1 h incubation		
	E. Coli Top 10	E.coli Top 10/ pBADgIIIAMRE not induced	E. coli Top 10 induced
4 h after induction	below detection	23ppm	329ppm

Example 7

Low throughput screening for inhibitors of the enzyme

Extracts of Ax 20 were prepared by mechanical disruption as described in Example 3. The extract (0.5 ml corresponding to 2 ml initial cell culture) was added to 3.5 ml of Buffer A and

amended with 40 µl of substrate (Nα-lauroyl-L-glutamine, 5% stock solution in methanol). Parallel samples were additionally amended with potential inhibitory compounds to give a final concentration of 0.5 and 5 mM. The samples were incubated for 2 h and then extracted with MTBE and HCl and analysed for released lauric acid using capillary GC. By comparing the samples containing potential inhibitory compounds with control samples with enzyme and substrate only, the inhibition (%) was calculated. Table 4 gives the result for selected zinc chelating compounds. The same assay was also made either with purified enzyme from the wild-type strain (see example 3) or with extracts from E.coli Top 10/pBADgIII AMRE induced with arabinose (see example 6). Furthermore, the same assay was made using stationary phase living cells of Ax20 instead of an isolated enzyme preparation. In this case successful uptake of the inhibitors into the cells and inhibition are measured simultaneously.

Table 4. Inhibition by zinc chelators of the isolated enzyme and of the enzymatic activity in intact cells

	% inhibition of enzyme activity in living cells	% inhibition of the isolated enzyme	
	5 mM	5 mM	0.5 mM
o-phenantrolin	90.3%	100 %	100%
2,2'-bipyridyl	n.d.	65%	n.d.
Aminotri(methylene-phosphonic acid)	55.7%	76.6%	n.d.
Ethylen-diimino-dibutyric acid	53.7%	44.3%	n.d.
Ethylendiamine-2-2'-diacetic acid	58.3%	100%	n.d.
Pyridine-2,6dicarboxylic acid	64.3%	100%	n.d.
N,N,N',N'-tetrakis-(2-pyridylmethyl)-ethylendiamine	85.2%	87.9%	n.d.
Dithiothreitol	n.d.	100%	98%

Example 8

High throughput screening for inhibitors of the enzyme

Potential inhibitory compounds were dissolved in Buffer A and aliquots of the solutions of different inhibitors (10µl) were distributed to individual wells of a white microtiter plate. Purified enzyme obtained from the strain E.coli Top 10/pBADgIII AMRE was diluted in Buffer A (200pg/ml final concentration) and added to the inhibitory compounds. After 10 min preincubation, the substrate (Nα-lauroyl-L-glutamine) was added to the individual wells to a final concentration of 0.05 mM. After 15 min of incubation, the amino-group of the released L-glutamine was derivatised by adding to each well of the microtiter plate 50µl of a fluorescamine

stock solution (2.5mM in acetonitrile; fluorescamine obtained from Fluka, Buchs, Switzerland). After 5 min the fluorescence in the wells of the microtiter plates was measured with an excitation wavelength of 360 nm and an emission wavelength of 460 nm. The fluorescence of control wells with enzyme, substrate and DMSO only was then compared to the fluorescence in wells containing potential inhibitors. By varying the inhibitor concentration, the IC50 value for each compound was determined, and the Ki values were calculated.

Table 5: Ki values for compounds of formula (I)

Compound	Ki value (nM)
5a	54 ± 1
5b	130 ± 10
5c	410 ± 20
8a	50 ± 3
8b	58 ± 4
8c	110 ± 10

Synthesis Example 1

The following description is made with reference to Scheme 1 in Figure 1

Synthesis of the thiol inhibitors

Step 1 - Synthesis of (2R)-2-Bromo-alkyl carboxylic acids (1)

In a synthesis based on Fisher, S.R.W.; *Justus Liebigs Ann.Chem.*, **1957**, 357 , (0.165 mol) of the corresponding D-α-aminoacid are solubilised in 165 mL HBr 48% and 150 mL water. The reaction mixture is cooled to 0°C and a solution of $NaNO_2$ (18.3g , 1.6 eq) in 60 mL water is added dropwise. The mixture is stirred for 2.5h at room temperature, then concentrated to remove the acid vapour, extracted with Et_2O four times. The organic layers are washed with water, NaCl sat., dried over Na_2SO_4, and concentrated under reduced pressure yielded compound **1** as oil used without further purification.

1a, R: $PhCH_2$: Yield 100%

Rf : 0.43 (CH_2Cl_2/MeOH/AA 9/1/0.2)

^1H NMR ($CDCl_3$ 270MHz) : 7.3(m, 5H), 4.3(t, 1H), 3.1-3.5 (m,2H).

1b, R: iBu: Yield 92.6%

Rf : 0.50 (CH_2Cl_2/MeOH/AA 9/1/0.5)

^{1}H NMR ($CDCl_3$ 270MHz) : 4.35(t, 1H), 2.0(t,2H), 1.8(m, 1H), 1.0(m, 6H)

1c, R: nBu: Yield: 100%

Rf : 0.48 (CH_2Cl_2/MeOH/AA 9/1/0.5)

^{1}H NMR ($CDCl_3$ 270MHz) : 4.3(t, 1H), 2.1-1.8 (m,2H), 1.5 (m, 4H), 0.9 (t,3H).

Step 2 - Synthesis of (2S)-2-Acetylsulfanyl alkyl carboxylic acids (2)

Compound **1** (0.165 mol) solubilised in 165 mL NaOH 1N (1eq) is cooled at 0°C. Potassium thioacetate (22.65g, 0.198 mol, 1.2 eq) in 60 mL H_2O is added dropwise and the reaction mixture is stirred for 16h at room temperature. The preparation is acidified by addition of HCl 1N (pH 1-2) then extracted with AcOEt three times. The organic layers are washed with water, NaCl sat., dried over Na_2SO_4, and concentrated under reduced pressure yielded compound **2** as orange oil used without further purification.

2a, R: $PhCH_2$: Yield 96.0%

Rf : 0.43 (Cyclohexane/AcOEt/AA 5/5/0.1)

HPLC Kromasil C18 5µ100A, 250x4.6 mm, CH_3CN-H_2O (0.05 % TFA) 40-60 R_t = 8.93 min

^{1}H NMR ($CDCl_3$ 270MHz) : 7.3(m, 5H), 4.3(t, 1H), 3.1-3.5 (m,2H), 2.2(s, 3H).

2b, R: iBu: Yield 91.0%

^{1}H NMR ($CDCl_3$ 270MHz): 4.2(t, 1H), 2.4(s,3H), 1.9-1.5(m, 4H), 0.9(m, 6H)

2c, R: nBu: Yield: 94.5%

^{1}H NMR ($CDCl_3$ 270MHz) : 4.1(t, 1H), 2.3(s, 3H), 1.9(m,1H), 1.65(m,1H), 1.3(m, 4H), 0.9 (t,3H).

Step 3 - Synthesis of N-[(2S)-2-acetylsulfanyl alkanoyl]-(S)-glutamine *tert*-butyl ester (3)

Compound **2** (2.607 mmol), (S)-Glutamine tert-butyl ester hydrochloride (1.2 eq, 746 mg), EDCI (1.2 eq, 929 mg), HOBt (1.2 eq, 479mg), Et_3N (1.2 eq, 438 µL) are stirred overnight in 10 mL THF/$CHCl_3$. The reaction mixture is concentrated under reduced pressure and diluted in H_2O/AcOEt.. The organic layer is washed with $NaHCO_3$ sat. (2x), citric acid 10% (2x), NaCl sat., dried over Na_2SO_4, and concentrated.

The crude product is purified by HPLC Kromasil C18 5µ100A, 250x20mm (CH_3CN/H_2O 0.05% TFA 40-60) yielded compound **3** as a solid.

3a, R: $PhCH_2$: Yield 32.4%, wt: 343mg.

HPLC Kromasil C18 5µ100A, 250x4.6 mm, CH_3CN-H_2O (0.05 % TFA) 50-50 R_t = 7.88 min

^{1}H NMR ($CDCl_3$ 270MHz) : 7.3-7.2(m, 5H), 4.4(m, 1H), 4.3(t, 1H), 3.2 (dd,1H), 2.8 (dd,1H), 2.2(s, 3H), 2.1(m,2H), 1.8(m,2H), 1.4(s, 9H).

3b, R: iBu: Yield 74.3%, wt: 352 mg.

HPLC Kromasil C18 5μ100A, 250x4.6 mm, CH_3CN-H_2O (0.05 % TFA) 50-50 R_t = 6.48 min

^1H NMR ($CDCl_3$ 270MHz) : 6.8(d, 1H), 4.4(m, 1H), 4.15(t, 1H), 2.4(s,3H), 2.3-1.5(m, 7H), 1.4(s, 9H), 0.9(m, 6H)

3c, R: nBu: Yield: 80.7%, wt: 307mg.

HPLC Kromasil C18 5μ100A, 250x4.6 mm, CH_3CN-H_2O (0.05 % TFA) 60-40 R_t = 6.75 min

^1H NMR ($CDCl_3$ 270MHz) : 6.8(d, 1H), 4.4(m, 1H), 4.1(t, 1H), 2.4(s, 3H), 2.2-1.5(m,10H), 1.4(s, 9H), 0.9 (t,3H).

Step 4 - Synthesis of N-[(2S)-2-acetylsulfanyl alkanoyl]-(S)-glutamine (4)

Compound 3 (0.58 mmol) is solubilized in 3 mL CH_2Cl_2 and 3 mL TFA are added at 0°C. The reaction mixture is stirred for 3h at room temperature. The solvent and excess reagent are eliminated under reduced pressure. The crude product is coevaporated 2 times with cyclohexane yielded compound 4 as oil used without further purification.

4a, R: $PhCH_2$: Yield 100%, wt: 206 mg.

HPLC Kromasil C18 5μ100A, 250x4.6 mm, CH_3CN-H_2O (0.05 % TFA) 30-70 R_t = 8.67 min

^1H NMR (DMSO+TFA 270MHz) : 8.5 (d, 1H), 7.2(m, 5H), 4.4(t, 1H), 4.05(m, 1H), 3.2 (dd,1H), 2.8 (dd,1H), 2.2(s, 3H), 2.1(t,2H), 1.9(m,1H), 1.8(m, 1H).

4b, R: iBu: Yield 100%, wt: 299 mg.

HPLC Kromasil C18 5μ100A, 250x4.6 mm, CH_3CN-H_2O (0.05 % TFA) 30-70 R_t = 6.36 min

^1H NMR (DMSO+TFA 270MHz) : 8.5(d, 1H), 4.3-4.0(m, 2H), 2.4(s,3H), 2.1(m, 2H)1.9(m, 1H), 1.7(m, 2H), 1.5(m, 1H), 1.3(m, 1H), 0.9(d, 3H), 0.8(d,3H)

4c, R: nBu: Yield: 100%, wt: 261mg.

HPLC Kromasil C18 5μ100A, 250x4.6 mm, CH_3CN-H_2O (0.05 % TFA) 30-70 R_t = 6.75 min

^1H NMR (DMSO+TFA 270MHz) : 8.5(d, 1H), 4.1(m, 2H), 2.4(s, 3H), 2.1(t,2H), 1.9(m, 1H), 1.7(m, 1H), 1.6(m, 1H), 1.2(m, 3H), 0.9 (t,3H).

Step 5 - Synthesis of N-[(2S)-2-mercapto alkanoyl]-(S)-glutamine (5)

Compound 4 (0.38 mmol) is solubilized under argon in 2 mL degassed MeOH and 1.16 mL degassed NaOH (3 eq) are added. The reaction mixture is stirred for 2h at room temperature. HCl 1N is added to obtain pH=1 and the solvent is eliminated under reduced pressure. The product is extracted with AcOEt. After evaporation the product is solubilized in water and lyophilised to give 5 as a white hygroscopic solid.

5a, R: $PhCH_2$: Yield 76.5%, wt: 91 mg.

HPLC Kromasil C18 5μ100A, 250x4.6 mm, CH_3CN-H_2O (0.05 % TFA) 30-70 R_t = 6.65 min

SM-ES(+): $[M+Na]^+ = 333$

^1H NMR (DMSO+TFA 270MHz) : 8.5 (d, 1H), 7.2(m, 5H), 4.1(m, 1H), 3.6(q, 1H), 3.1 (dd,1H), 2.7 (dd,1H), 2.1(t, 2H), 1.9(m,1H), 1.8(m, 1H).

5b, R: iBu: Yield 51.3%, wt: 133 mg.

SM-ES(-): $[M-H]^- = 275$

HPLC Kromasil C18 5μ100A, 250x4.6 mm, CH_3CN-H_2O (0.05 % TFA) 30-70 $R_t = 5.36$ min

^1H NMR (DMSO+TFA 270MHz) : 8.3(d, 1H), 4.1(m, 1H), 3.4(m, 1H), 2.1(m, 2H), 2.0-1.3(m, 5H), 0.9(d, 3H), 0.8(d,3H)

5c, R: nBu: Yield: 74.3%, 168 mg.

HPLC Kromasil C18 5μ100A, 250x4.6 mm, CH_3CN-H_2O (0.05 % TFA) 30-70 $R_t = 5.89$ min

SM-ES(+): $[M+Na]^+ = 299$

^1H NMR (DMSO+TFA 270MHz) : 8.2(d, 1H), 4.2(m, 1H), 3.2 (q, 1H), 2.1(t,2H), 1.9(m, 1H), 1.7(m, 1H), 1.6(m, 1H), 1.2(m, 3H), 0.8 (t,3H).

Synthesis Example 2

This synthesis is described with reference to Scheme 2 of Figure 2 :

Synthesis of the phosphinic inhibitors

Step 1 - Synthesis of Alkyl phosphinic acids (6)

The synthesis is based on the method of Boyd, E.A.; Regan, A.C.; *Tetrahedron Letters*, **1994**, 24, 4223. In a 100mL flask equipped with a septum and a condenser, 4.2g (51.85 mol) ammonium phosphinate and HMDS (8.57g, 53.08 mmol, 1.02 eq) are heated under N_2 at 100-110°C for 2h. The reaction mixture is cooled at 0°C and 50mL dried CH_2Cl_2 is added followed by the addition of the bromide derivative (53.08 mmol, 1.02 eq). The mixture is stirred overnight at room temperature.

The precipitate is filtered and the filtrate concentrated under reduced pressure. The crude product is dissolved in CH_2Cl_2/MeOH. The precipitate is removed and the crude product is eluted on silica gel (CH_2Cl_2/MeOH/AA 9/1/0.4) yielding compound **6**.

6a, R: $PhCH_2$: Yield 43.3%, wt: 3.50 g.

Rf : 0.21 (CH_2Cl_2/MeOH/AA 9/1/0.4)

^1H NMR (DMSO+TFA 270MHz) : 7.2(m, 5H), 6.9(d, 1H), 3.1(dd, 2H)

6b, R: iBu: Yield 32.1%, wt: 2.86 g.

^1H NMR (DMSO+TFA 270MHz) : 6.9(d, 1H), 3.6(m, 2H), 1.5(m, 1H), 0.9(m, 6H)

6c, R: nBu: Yield: 56.9%, wt: 3.60 g.

^{1}H NMR (DMSO+TFA 270MHz) : 6.9(d, 1H), 1.6(m, 2H), 1.3(m, 4H), 0.8(t, 3H)

Step 2 - Synthesis of 2-(Benzyl-hydroxy-phosphinoylmethyl)-4-(trityl-carbamoyl)-butyric ethyl ester (7a)

This synthesis is based on the method of Boyd, E.A.; Regan, A.C.; *Tetrahedron Letters*, **1994**, 24, 4223. In a 25mL flask equipped with a septum and a condenser, compound **6a** (156 mg, 1 mmol) and HMDS (218μL, 1.02 eq) are warmed up under N_2 at 100-110°C for 2h. The reaction mixture is cooled at 0°C and compound **14** (426 mg, 1.03 mmol) in 5mL dried CH_2Cl_2 is added. The mixture is heated overnight at 60°C.

The reaction mixture is concentrated under reduced pressure. The crude product is purified by HPLC Kromasil C18 5μ100A, 250x20mm (CH_3CN/H_2O 0.05% TFA 60-40) yielded 234mg compound **7a** as a white solid. Yield 41.1%.

HPLC Kromasil C18 5μ100A, 250x4.6 mm, CH_3CN-H_2O (0.05 % TFA) 70-30 R_t = 5.99 min

^{1}H NMR (DMSO+TFA 270MHz) : 8.5 (s, 1H), 7.2(m, 20H), 4.1(q, 2H), 3.0(d, 2H), 2.6(m, 1H), 2.3(t, 2H), 2.0-1.6 (m, 4H), 1.1(t, 3H)

Synthesis of 2-(Alkyl-hydroxy-phosphinoylmethyl)-4-(trityl-carbamoyl)-butyric ethyl ester (7b-c)

Compound **6b** or **6c** (3.27 mmol) is solubilized in 3mL CH_3CN. Compound **14** (1.35 g, 3.27 mmol) and BSA (4.06 mL, 5eq) are added and the reaction mixture is stirred 72h at room temperature under N_2. The reaction mixture is concentrated under reduced pressure. The crude product is purified by HPLC Kromasil C18 5μ100A, 250x20mm (CH_3CN/H_2O 0.05% TFA 60-40).

7b, R: iBu: Yield 5.3%, wt: 92 mg, white solid.

HPLC Kromasil C18 5μ100A, 250x4.6 mm, CH_3CN-H_2O (0.05 % TFA) 70-30 R_t = 5.57 min

^{1}H NMR (DMSO+TFA 270MHz) : 8.6 (s, 1H), 7.2(m, 15H), 4.0(q, 2H), 2.6(m, 1H), 2.2(t, 2H), 1.9 (m, 2H), 1.7(m, 3H), 1.5 (m, 2H), 1.1(t, 3H), 0.9(d, 6H)

7c, R: nBu: Yield: 8.9%, wt: 155 mg, white solid..

HPLC Kromasil C18 5μ100A, 250x4.6 mm, CH_3CN-H_2O (0.05 % TFA) 60-40 R_t = 10.29 min

^{1}H NMR (DMSO+TFA 270MHz) : 7.2(m, 15H), 6.8(s, 1H), 4.1(q, 2H), 2.7(m, 1H), 2.4-1.4(m, 12H), 1.2(t, 3H), 0.9(d, 3H)

Step 3 - Synthesis of 2-(Alkyl-hydroxy-phosphinoylmethyl)-4-(trityl-carbamoyl)-butyric acid (8)

Compound 7 (0.41 mmol) is solubilized in 2mL EtOH and 2mL LiOH 1N(5eq) are added. The reaction mixture is stirred for 2h at room temperature. HCl 1N is added to obtain pH=1 and EtOH is removed under reduced pressure. The product is extracted by AcOEt. The organic layers are washed with NaCl sat., dried over Na_2SO_4 yielding compound **8** as oils.

8a, R: $PhCH_2$: Yield 95.0%, wt: 211 mg.

HPLC Kromasil C18 5μ100A, 250x4.6 mm, CH_3CN-H_2O (0.05 % TFA) 40-60 R_t = 7.70 min

^{1}H NMR (DMSO+TFA 270MHz) : 8.5 (s, 1H), 7.2(m, 20H), 3.0(dd, 2H), 2.5(m, 1H), 2.3-1.6 (m, 6H)

8b, R: iBu: Yield 97.6%, wt: 81 mg.

HPLC Kromasil C18 5μ100A, 250x4.6 mm, CH_3CN-H_2O (0.05 % TFA) 60-40 R_t = 5.46 min

^{1}H NMR (DMSO+TFA 270MHz) : 8.6 (s, 1H), 7.2(m, 15H), 2.6(m, 1H), 2.2(m, 2H), 1.9 (m, 2H), 1.7(m, 3H), 1.5(m, 2H), 0.9(d, 6H)

8c, R: nBu: Yield: 69.2%, wt: 101 mg.

HPLC Kromasil C18 5μ100A, 250x4.6 mm, CH_3CN-H_2O (0.05 % TFA) 70-30 R_t = 3.92 min

^{1}H NMR (DMSO+TFA 270MHz) : 8.6(s, 1H), 7.2(m, 15H), 2.7(m, 1H), 2.0-1.0(m, 12H), 0.8(d, 3H)

Step 4 - Synthesis of 2-(Alkyl-hydroxy-phosphinoylmethyl)-4 -carbamoyl-butyric acid (9)

Compound **8** (0.197 mmol) is solubilized in 4mL TFA in presence of 90 μL iPr_3SiH. The reaction mixture is stirred 2h at room temperature. Excess TFA is removed under reduced pressure and the reaction mixture is co-evaporated with cyclohexane (2x). The crude product is purified by HPLC Kromasil C18 5μ100A, 250x20mm (CH_3CN/H_2O 0.05% TFA 30-70) yielding compound **9**.

9a, R: $PhCH_2$: Yield 88.1%, wt: 52 mg, oily product.

SM-ES(+): $[M+H]^+ = 300$

HPLC Kromasil C18 5μ100A, 250x4.6 mm, CH_3CN-H_2O (0.05 % TFA) 50-50 R_t = 2.34 min

^{1}H NMR (DMSO+TFA 270MHz) : 7.2(m, 5H), 3.0(d, 2H), 2.5(m, 1H), 2.0-1.6 (m, 6H)

9b, R: iBu: Yield 71.5%, wt: 30 mg, oily product.

SM-ES(-): $[M-H]^- = 264$

HPLC Kromasil C18 5μ100A, 250x4.6 mm, CH_3CN-H_2O (0.05 % TFA) 60-40 R_t = 2.36 min

^{1}H NMR (DMSO+TFA 270MHz) : 2.6(m, 1H), 2.0-1.5(m, 9H), 0.9(d, 6H)

9c, R: nBu: Yield: 98.0%, wt: 51mg, oily product.

SM-ES(-): $[M-H]^-$ = 264

HPLC Kromasil C18 5μ100A, 250x4.6 mm, CH_3CN-H_2O (0.05 % TFA) 70-30 R_t = 3.92 min

^1H NMR (DMSO+TFA 270MHz) : 2.5(m, 1H), 2.1-1.1(m, 12H), 0.8(d, 3H).

Synthesis Example 3

This synthesis is described with reference to Scheme 3 of Figure 3.

Synthesis of the Ethyl 2[2-(N-trityl)carboxamido ethyl)] acrylate

Step 1 - Synthesis of Diethyl 2-(2-*tert*-butyloxycarbonyl ethyl) malonate (10)

In a method based on Prabhu, K. R.; Pillarsetty, N.; Gali, H.; Katti, K. V.; *J.Am.Chem.Soc.*, **2000**, 122, 1554, a mixture of diethylmalonate (11.12g, 10.55 mL, 69.46 mmol), tert-Butylacrylate (10.17 mL, 69.46 mmol, 1eq), K_2CO_3 (9.60 g, 1eq), nBu_4NHSO_4 (258 mg 0.01 eq) in 40 mL toluene are heated under reflux during 16h. The reaction mixture is filtered, concentrated under vacuum yielded 19.6g compound **10** as oil used without further purification.

Yield: 98.0%

HPLC Kromasil C18 5μ100A, 250x4.6 mm, CH_3CN-H_2O (0.05 % TFA) 70-30 R_t = 8.72 min

^1H NMR ($CDCl_3$ 270MHz) : 4.1 (q, 4H), 3.3 (t, 1H), 2.2 (m, 2H), 2.05 (m, 2H), 1.3 (s, 9H), 1.1 (m, 6H).

Step 2 - Synthesis of Diethyl 2-(2-carboxyethyl) malonate (11)

To a solution of compound **10** (19.6g, 68.05 mmol), in 400 mL CH_2Cl_2 is added 400mL of TFA. The mixture is stirred under during 48h at room temperature. The reaction mixture is concentrated under vacuum, coevaporated two times with cyclohexane to eliminate excess TFA yielded 15.8g compound **11** as oil used without further purification.

Yield: 100.0%

^1H NMR ($CDCl_3$ 270MHz) : 8.7(s, 1H), 4.2 (q, 4H), 3.4 (t, 1H), 2.5 (m, 2H), 2.1 (m, 2H), 1.2 (m,6H).

Step 3 - Synthesis of Diethyl 2-(2-N-tritylcarboxamidoethyl) malonate (12)

In a method based on Haynes, R.K.; Starling, S.M.; Vonwiller, S.C.; *J.Org.Chem.*, **1995**, 60, 4690, compound **11** (15.79g, 68.10 mmol) in 12 mL thionyl chloride is heated under reflux during 1h. The reaction mixture is concentrated under vacuum, dissolved in 20 mL CH_2Cl_2, then a solution of trityl amine (28.3g 88.52 mmol) and Et_3N in 20 mL CH_2Cl_2 is added dropwise. The reaction mixture is stirred for 48h at room temperature.

The reaction is stopped by addition of saturated solution of K_2CO_3 and the desired product extracted by Et_2O.

The organic layer is washed with K_2CO_3 sat., HCl 2M, dried over Na_2SO_4 , and concentrated under reduced pressure. The crude product is eluted on silica gel (elution CHex/AcOEt 6/4) yielded 20.9g of the desired compound **12** as a white solid.

Yield: 65.0%

Mp: 102-104°C

TLC (CHex/AcOEt 6/4) Rf:0.56

HPLC Kromasil C18 5μ100A, 250x4.6 mm, CH_3CN-H_2O (0.05 % TFA) 70-30 R_t = 12.45 min

^{1}H NMR ($CDCl_3$ 270MHz) : 7.4-7.1(m, 15H), 6.6(s, 1H), 4.15 (q, 4H), 3.4 (t, 1H), 2.35 (t, 2H), 2.1 (q, 2H), 1.3 (t,6H).

Step 4 - Synthesis of Monoethyl 2-(2-N-tritylcarboxamidoethyl) malonate (13)

To a solution of compound **12** (20.93g, 44.41 mmol) in 80 mL EtOH at 0°C is added KOH (2.53g 1.025 eq) in 100 mL EtOH. The reaction mixture is stirred for 48 h at 4°C. The reaction mixture is concentrated under reduced pressure. The mixture is dissolved in water, extracted by Et_2O. The aqueous layer is acidified with HCl 3M. The precipitate is filtered, dried, given 16.4g compound **13** as a white solid.

Yield: 65.0%

Mp: 122-124°C

TLC (CHex/AcOEt 6/4) Rf:0.56

HPLC Kromasil C18 5μ100A, 250x4.6 mm, CH_3CN-H_2O (0.05 % TFA) 70-30 R_t = 12.45 min

^{1}H NMR ($CDCl_3$ 270MHz) : 7.4-7.1(m, 15H), 6.6(s, 1H), 4.15 (q, 2H), 3.4 (t, 1H), 2.35 (t, 2H), 2.1 (q, 2H), 1.3 (t,3H).

Step 5 - Synthesis of Ethyl 2[2-(N-trityl)carboxamido ethyl)] acrylate (14)

Et_2NH (3.80 mL, 36.85 mmol), 37% sol. Formaldehyde (4.5 mL,1.5 eq) are mixed with compound **13** (16.4g 36.85 mmol) and stirred 48 h at room temperature. The reaction mixture is taken up with 210 mL of a mixture H_2O/Et_2O. The aqueous layer is extracted two times with Et_2O. The organic layers are washed with citric acid 10%, H_2O, $NaHCO_3$ sat., NaCl sat., dried over Na_2SO_4, and concentrated under reduced pressure yielded compound **14** (11.71g) as a white solid.

Yield: 79.6%

Mp: 120-122°C

HPLC Kromasil C18 5μ100A, 250x4.6 mm, CH_3CN-H_2O (0.05 % TFA) 70-30 R_t = 11.81 min
^1H NMR ($CDCl_3$ 270MHz) : 7.4-7.1(m, 15H), 6.6(s, 1H), 6.1 (s,1H), 5.6 (s, 1H), 4.15 (q, 2H), 2.6 (t, 2H), 2.4 (t, 2H), 1.3 (t,3H).

INTERNATIONAL FORM

Givaudan SA
1214 Vernier
Switzerland

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the DEPOSITOR:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
Ax 20	DSM 14267

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I. above was accompanied by:

(X) a scientific description
(X) a proposed taxonomic designation

(Mark with a cross where applicable).

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on 2001-04-26 (Date of the original deposit)[1].

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): U. Wirths
Address: Mascheroder Weg 1b D-38124 Braunschweig	Date: 2001-04-30

Where Rule 6.4 (d) applies, such date is the date on which the status of international depositary authority was acquired.

Form DSMZ-BP/4 (sole page) 0196

Claims

1. An isolated N_α-acyl-glutamine-aminoacylase.

2. An enzyme comprising the amino acid sequence set forth in SEQ ID NO: 1

3. An enzyme encoded for by nucleic acid comprising a nucleotide sequence set forth in SEQ ID NO: 5.

4. An isolated nucleic acid encoding for an enzyme comprising the amino acid sequence of SEQ ID NO: 1.

5. An isolated nucleic acid according to claim 4 comprising a nucleotide sequence set forth in SEQ ID NO: 5.

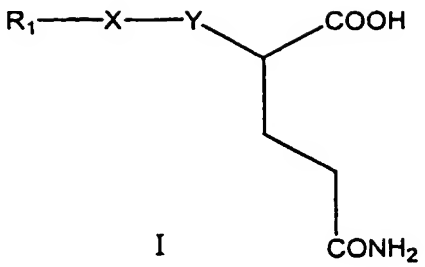
6. An expression vector comprising nucleic acid encoding for the amino acid sequence of SEQ ID NO: 1.

7. An expression vector according to claim 6 comprising nucleic acid comprising a nucleotide sequence set forth in SEQ ID NO: 5

8. Host cells transformed with expression vectors according to claim 6 or claim 7.

9. A method for the production of an enzyme as defined in any of the claims 1 to 3 which comprises culturing a host cell having incorporated therein an expression vector encoding for the enzyme, under conditions sufficient for expression of said enzyme in said host cell, thereby causing the production of an expressed enzyme; and recovering the enzyme produced by the cell.

10. Compounds of formula (I)



I

wherein

Y represents a direct bond to X, or a divalent chain that may contain carbon, oxygen or nitrogen atoms,

X is a zinc-chelating group, and

R_1 is a linear, branched or cyclic carbon chain having about 1 to 14 carbon atoms, optionally containing one or more heteroatoms such as O, N or S, or unsaturation, the chain optionally supports one or more substituents selected from amide, ester, keto, ether, amine, halogen or hydroxyl, or aryl or heteroaryl groups, which aryl or heteroaryl groups optionally support one or more substituents selected from amide, ester, keto, ether, amine, halogen, alkyl or hydroxyl.

11. Compound according to claim 10 wherein Y is an amide group –CONH-, an alkylene group selected from methylene, ethylene or propylene, $-CH_2-NH-$, or –NH-.

12. Compound according to claim 10 or claim 11 wherein X is a methylene thiol group (II), a phosphinyl group (III), or a group bearing a carboxylic acid group.

13. Compound according to any of the claims 10 to 12 wherein the group R_1 is selected from n-butyl, sec-butyl, benzyl or phenylethyl.

14. Compound according to claims 10 to 13 wherein

Y represents an amide group –CONH- when X is methylene thiol (II), or

Y represents a methylene group when X is phosphinyl (III).

15. Inhibitor of an enzyme as defined in claims 1 to 3 selected from a compound as defined in any of the claims 10 to 14.

16. Composition comprising a body odour-suppressing quantity of a compound as defined in any of the claims 10 to 15.

17. Composition according to claim 16 wherein the compound is present in amounts of about 0.01 to 0.5 % by weight.

18. Composition according to claim 16 or claim 17 selected from cosmetic and personal care products, in particular deo-sticks, roll-ons, pump-sprays, aerosols, deodorant soaps, powders, solutions, gels, creams, sticks, balms and lotions.

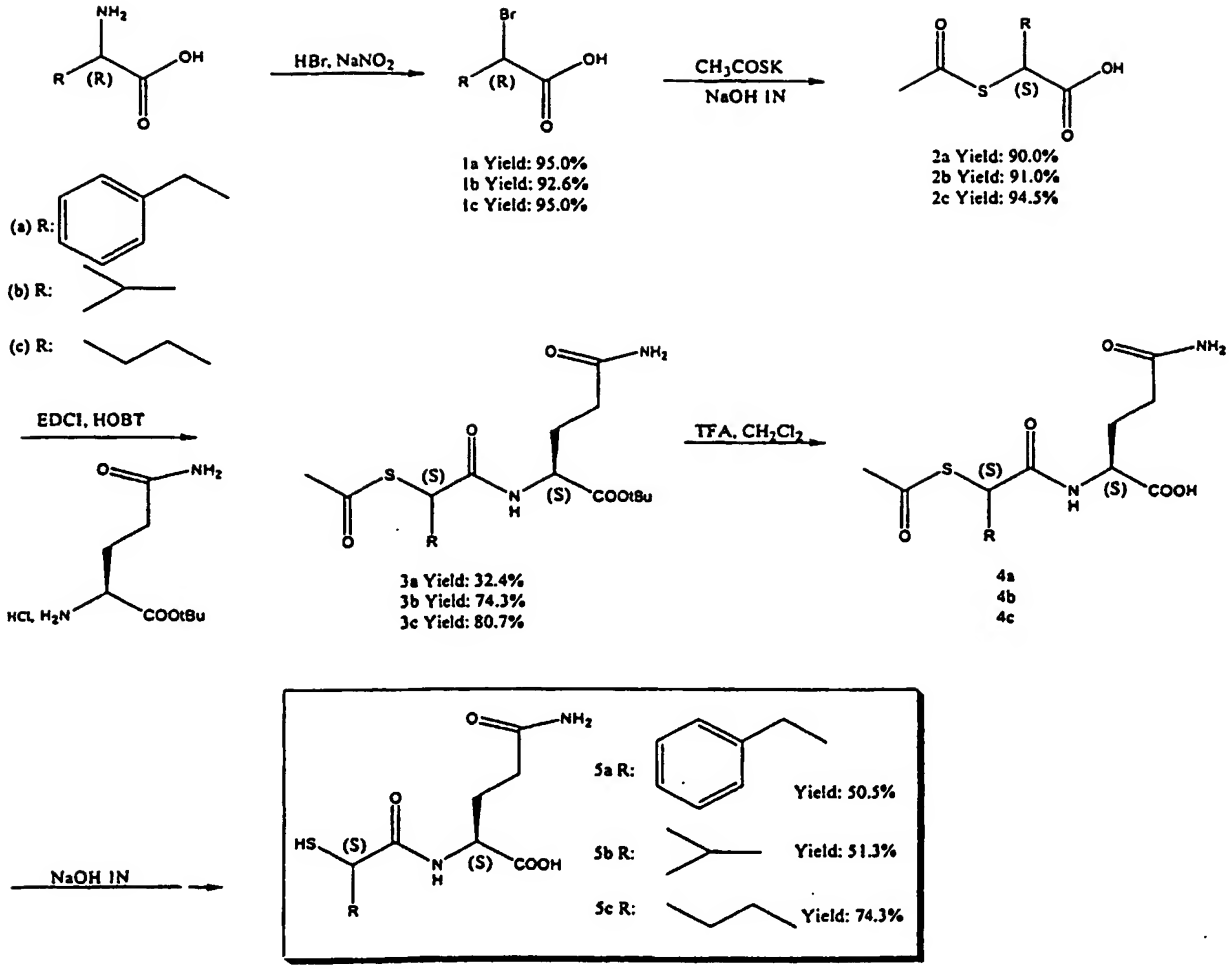
18. Use of a compounds or compositions according to any of the claims 10 to 18 to inhibit the enzyme defined by any of the claims 1 to 3 in its ability to cleave compounds contained in sweat into short-chained, branched fatty acids.

19. A method of screening compounds for inhibitor activity of an enzyme as defined in any of the claims 1 to 3 comprising the steps of I) incubating the enzyme, cells, or cell extract containing the enzyme with a substrate and a compound with potential inhibitory properties, and II) measuring release of malodorous compounds and/or free L-glutamine.

20. A method of suppressing axillary malodour comprising the step of providing a composition for application to a person in need of treatment, said composition containing an inhibitor compound and a dermatologically acceptable vehicle therefor, said compound being selected from a screening of compounds for activity in the inhibition of the enzyme.

Figure 1

Scheme 1



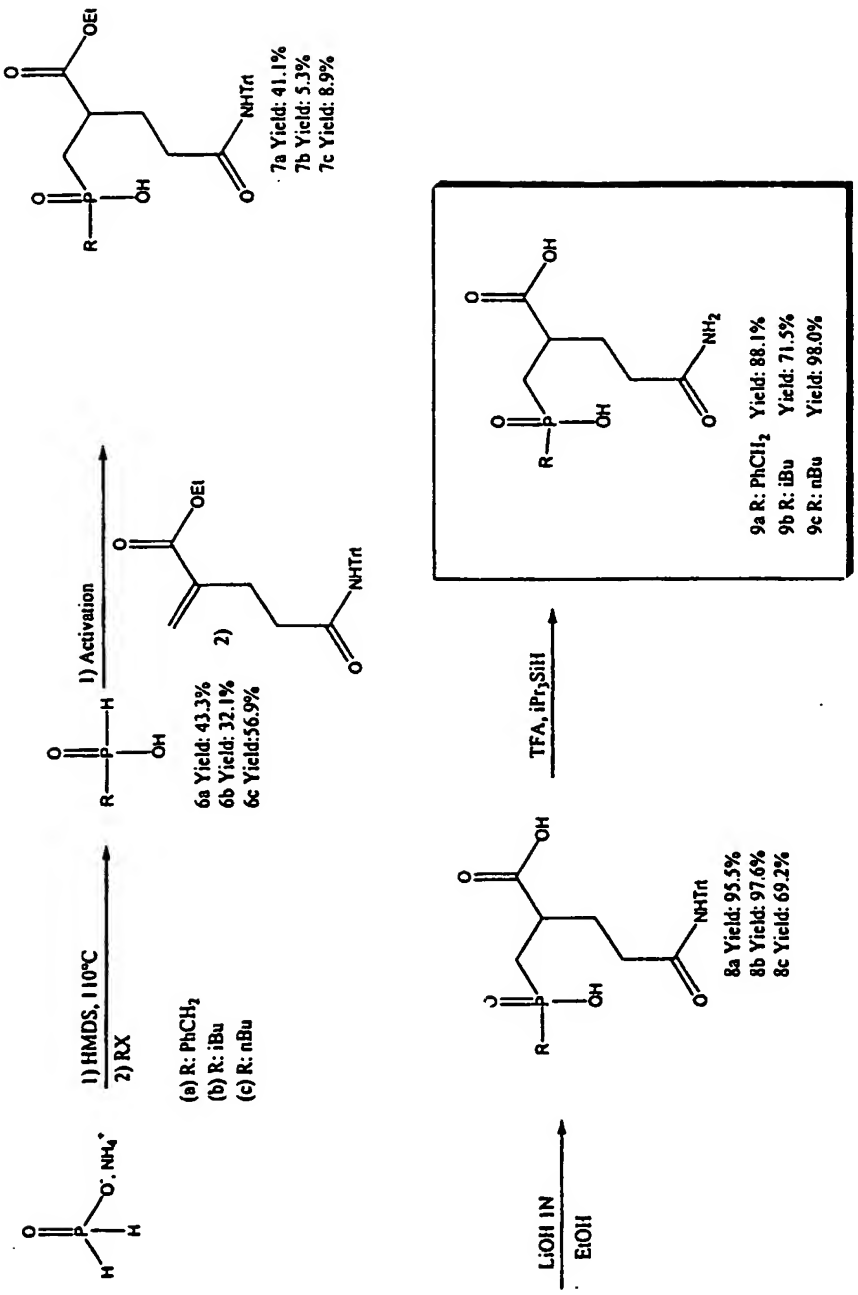
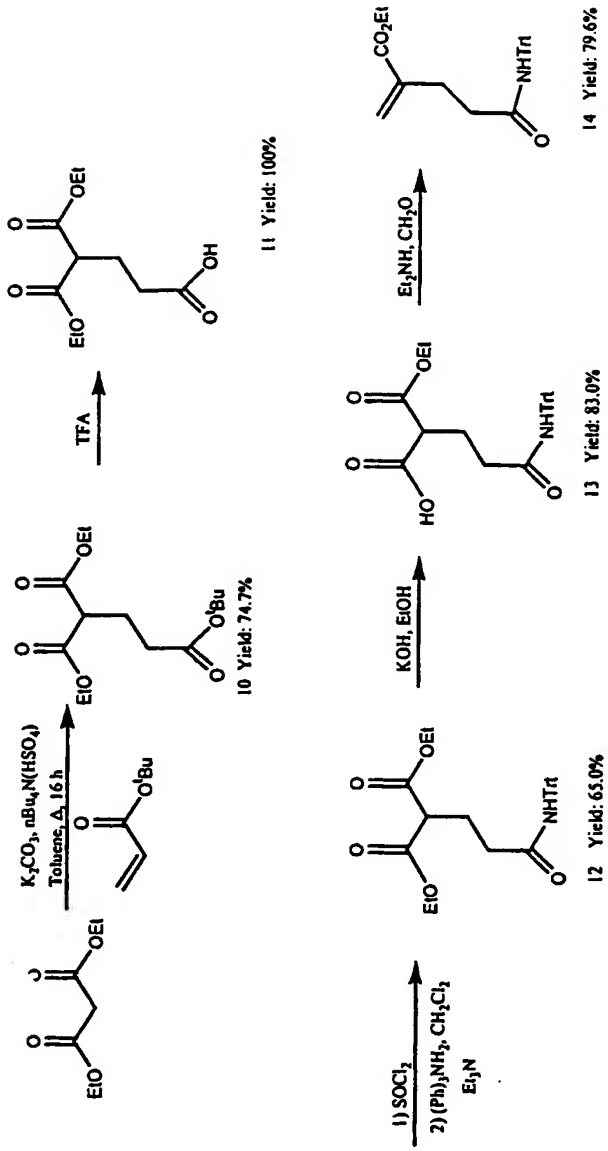


Figure 2



Scheme 3

Figure 3.

Sequence listing part of description

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Gly Gly Gly Gly Ala Arg His Met Val Asp Asp Gly Leu Ala Glu Lys
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Ile Ala Ala Pro Asp Val Cys Phe Ala Gln His Val Phe Asn Glu Asp
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ccc gcc ttt ggc tac gtg ttc acc ccc ggc cgg ttt cta acg gcg gcg          579
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Lys Leu Gln Thr Ile Val Ser Arg Glu Val Asp Pro Asn Glu Val Ala
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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/CH02/00262

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

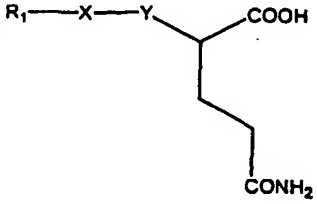
Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report:
16 October 2003

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/092024 A3

(54) Title: COMPOUNDS AND METHODS FOR INHIBITING AXILLARY MALODOUR



(I)

(57) Abstract: Enzymes mediating in the release of compounds characteristic of human malodour and in particular axillary malodour, and compounds that inhibit said enzymes - having general formula (I).

INTERNATIONAL SEARCH REPORT

International Application No
PCT/C[illegible]2/00262

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/55 C12N9/80 C12N5/10 G01N33/573 G01N33/68
C07C323/60 C07C323/32 C07F9/30 A61K7/32

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N G01N C07C C07F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

MEDLINE, EPO-Internal, PAJ, BIOSIS, WPI Data, CHEM ABS Data, SEQUENCE SEARCH, INSPEC, EMBL, BEILSTEIN Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	A. NATSCH, ET AL.: "A specific bacterial aminoacylase cleaves odorant precursors secreted in the human axilla." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 278, no. 8, 21 February 2003 (2003-02-21), pages 5718-5727, XP002233274 American Society of Biological Chemists, Baltimore, MD, US ISSN: 0021-9258 the whole document --- -/--	1-20

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 August 2003	27. 08. 2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	English, R

7

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	S. PITTELKOW, ET AL: "Human and porcine aminoacylase I overproduced in a baculovirus expression vector system: Evidence for structural and functional identity with enzymes isolated from kidney." PROTEIN EXPRESSION AND PURIFICATION, vol. 12, no. 2, March 1998 (1998-03), pages 269-276, XP002233275 Academic Press, San Diego, CA, US ISSN: 1046-5928 abstract; figure 5	1,9,19
X	WO 98 27201 A (HOECHST SCHERING AGREVO) 25 June 1998 (1998-06-25) SEQ ID NO: 3 claims; table 3	1,9,19
A	table 3	10,15, 16,18,20
A	D.B. GOWER, ET AL.: "Comparison of 16-androstene steroid concentrations in sterile apocrine sweat and axillary secretions: interconversions of 16-androstenes by the axillary microflora - a mechanism for axillary odour production in man?" JOURNAL OF STEROID BIOCHEMISTRY AND MOLECULAR BIOLOGY, vol. 48, no. 4, 1994, pages 409-418, XP000882081 Elsevier Science Publishers, Oxford, GB ISSN: 0960-0760 the whole document	1-9,19
A	WO 00 01355 A (UNILEVER, ET AL.) 13 January 2000 (2000-01-13) page 3, line 21 - line 26; example 1	1-9,19
A	DATABASE EMBL 'Online! 1 May 2000 (2000-05-01) retrieved from EBI Database accession no. Q9RKM4 XP002233278 abstract	1-9,19
A	C. FROEBE, ET AL.: "Axillary malodour production: a new mechanism" JOURNAL OF THE SOCIETY COSMETIC CHEMISTS, vol. 41, no. 3, 1990, pages 173-186, XP000993276 Society of Cosmetic Chemists, New York, NY, US ISSN: 0037-9832 the whole document	1-9,19

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	X.-N. ZENG, ET AL.: "Analysis of characteristic odours from human male axillae" JOURNAL OF CHEMICAL ECOLOGY, vol. 17, no. 7, 1991, pages 1469-1492, XP008008746 Kluwer Academic/Plenum Publishing, New York, NY, US ISSN: 0098-0331 the whole document	1-9,19
A	US 4 745 067 A (H. UMEZAWA, ET AL.) 17 May 1988 (1988-05-17) claims	1-9,19
X	EP 0 815 833 A (GIVAUDAN-ROURE (INTERNATIONAL)) 7 January 1998 (1998-01-07) page 5, line 19 - line 23; claims; examples 1,2	10,11, 15-18

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CH 02/00262

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 10-13 and 20 (in part)
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II *Observations where unity of invention is lacking (Continuation of item 2 of first sheet)*

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 10-13 and 20 (in part)

1. Present claims 10-13 relate to an extremely large number of possible compounds. Support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible.

Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the compounds in which Y is defined as in claim 11.

2. Furthermore, the present claims 10-13 relate to a product defined by reference to a desirable characteristic or property, namely that the compound is able to chelate to zinc via the substituent X.

The claims cover all products having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT for only a very limited number of such products. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible.

Independently of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the product by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the products where X is a methylene thiol group (CH2SH) or a phosphinyl group (P(=O)OH).

3. Present claim 20 relates to an extremely large number of possible methods. Support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the methods claimed. In the present case, the claim so lacks support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible.

Consequently, the search has been carried out for those parts of the claim which appear to be supported and disclosed, namely those parts relating to the methods involving compounds of claim 10 and the enzyme of claim 1.

The applicant's attention is drawn to the fact that claims, or parts of

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

XOCID: <WO__02092024A3_I_>

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-9 and 19

An isolated N-alpha-acetyl-glutamine-aminoacylase, nucleic acid encoding for it, vectors, host cells, methods to produce such enzyme, use of such enzyme to screen for inhibitory compounds.

2. Claims: 10-18 and 20

compounds according to formula I (claim 10), and their use to inhibit a N-alpha-acetyl-glutamine-aminoacylase and axillary malodour.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/[redacted]02/00262

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9827201 A	25-06-1998	DE 19652284 A1	18-06-1998
		AU 727831 B2	04-01-2001
		AU 5753598 A	15-07-1998
		BR 9714513 A	21-03-2000
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		WO 9827201 A2	25-06-1998
		EP 0942965 A2	22-09-1999
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		JP 2001506130 T	15-05-2001
		NZ 335852 A	29-06-2001
		PL 334530 A1	28-02-2000
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		NZ 328101 A	25-11-1998
		US 6150542 A	21-11-2000
		US 5925339 A	20-07-1999
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